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Glomerin and homoglomerin from the North American pill millipede *Onomeris sinuata* (Loomis, 1943) (Diplopoda, Pentazonia, Glomeridae)

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Abstract

Specimens of the North American glomerid millipede *Onomeris sinuata* (Loomis, 1943) were collected at the type locality in Alabama, USA, and maintained briefly in the laboratory in native leaf litter. The millipedes could not be induced to produce defensive secretions by rough handling, tapping, squeezing or leg-pinching. Four specimens were extracted in methanol and the extract analyzed using gas chromatography/mass spectroscopy. The analysis revealed the alkaloids glomerin and homoglomerin, previously reported as components in the defensive secretion of the European glomerid *Glomeris marginata* (Villers, 1789). This report is only the second for the occurrence of quinazolinone alkaloids in animals.

Keywords

Onomeris sinuata, *Glomeris marginata*, quinazolinone alkaloids, Alabama, USA

Introduction

Nearly simultaneously in 1966, Schildknecht et al. (1966) and Meinwald et al. (1966) announced the discovery of two quinazolinone alkaloids in the defensive secretion of the European millipede *Glomeris marginata* (Villers, 1789). Schildknecht et al. (1966, 1967) named one of these compounds glomerin; later Schildknecht and

Wenneis (1966) were able to associate the name glomerin with a specific structure: 1,2-dimethyl-4(3H)-quinazolinone, a result confirmed independently by Meinwald et al. (1966). The second compound, homoglomerin, is 1-ethyl-2-methyl-4(3H)-quinazolinone (Schildknecht and Wenneis 1967a). Up to the time of this study, *G. marginata* was the only known animal source of any quinazolinone alkaloid (Eisner et al. 2005). Schildknecht and Wenneis (1967b) established by radioactive tracer studies that *G. marginata* synthesizes quinazolinones from anthranilic acid and does not sequester them from plant sources.

Both compounds were found to be potent repellents of frogs, birds and mice (Schildknecht and Wenneis 1967a) and to act as antifeedants and sedatives to a lycosid spider (Carrell and Eisner 1984).

Carrell (1984) studied the production and storage of the secretion, which, in addition to its content of glomerin and homoglomerin, contains protienaceous components that make it very sticky, thus adding a physical dimension to its protection for the millipede. Following this 1984 publication, no further research on the occurrence of glomerin and homoglomerin was published. Summaries appear in Eisner et al. (1978, 2005).

Glomerid millipedes are capable of complete enrollment. The posteriormost body segment, the anal shield, is enlarged and hoodlike, and in the enrolled condition completely covers the head and much-reduced collum (first postcephalic segment), locking under the anterior margin of the second segment, while the semispherical, overlapping terga enclose the sides (Hopkin and Read 1992, and pers. obs. by WAS). Thus a potential predator such as a spider or ant is presented with a hard, glossily smooth, and nearly perfect sphere. However, large wolf spiders are capable of spanning small enrolled glomerids with their chelicerae, and biting them (Carrell and Eisner 1984), and one presumes that frogs, birds or mice could swallow such morsels whole. Despite the protection against some enemies provided by the hard exoskeleton and enrolling habit, Glomerida are the only order of the millipede subclass Pentazonia which additionally possess chemical defenses (Sierwald and Bond 2007). Beginning on the third segment and continuing posterior for eight segments (to the antepenultimate), *Glomeris* species bear dorsomedian pores (the so-called ozopores) from which empty the products of paired, or two-armed repugnatorial glands; these glands produce the sticky secretion that also contains glomerin and homoglomerin. The secretion is expelled by muscles wound around the glands, through a muscular valve (Eisner et al. 1978). In keeping with this potent chemical weapon, many species of *Glomeris* are aposematically colored, with transverse bands or spots of bright yellow, orange or red (Blower 1958, Demange 1981, pers. obs. by WAS).

While a prominent feature of the soil fauna of much of Europe, only two of the 34 described glomerid genera are found in America, where they appear at present to be limited to the southern Appalachian Mountains (*Onomeris*) and to the San Francisco Bay area of California, southern México, and Guatemala (*Glomeroides*) (Shear 1986, Hoffman 1999). *Onomeris sinuata* was originally described by Loomis (1943) from Monte Sano State Park, Alabama, as *Trichomeris sinuata*; *Trichomeris* was synonymized with *Onomeris* by Wesener (2010). Unlike European *Glomeris* species, species of *Ono-*

meris and *Glomeroides* are small (4–10 mm long), inconspicuous, dun-colored inhabitants of soil and deciduous leaf litter, lacking any aposematic coloration. A number of *Glomeroides* species have so far been recorded only from Central American and Méxican caves (Shear 1986). Neither chemical defense nor ozopores have been previously demonstrated in any North American species.

Methods

Specimens of *Onomeris sinuata* were collected by TW at the type locality, Monte Sano State Park, Alabama, under a permit issued by the state of Alabama. Specimens were placed in containers with damp leaf litter from the collection site, transported to the Field Museum of Natural History in Chicago and subsequently to the laboratory of WAS in Hampden-Sydney, Virginia. There, the millipedes were subjected to various physical stresses designed to elicit secretion and finally were live-extracted in small quantities of methanol in glass vials with Teflon-lined caps. The vials were sent to the laboratory of THJ at the Virginia Military Institute, Lexington, Virginia, for analysis.

Gas Chromatograph/Mass Spectroscopy analysis of the methanol extract was carried out using a Shimadzu model 2010 GC/MS equipped with an RTX-5, 30 m 3 0.25-mm i.d. The specimens are now preserved in 70% ethanol and eventually will be deposited as vouchers in the collection of the Field Museum.

Additional specimens (FMNH-INS 56316) preserved in 95% ethanol were carried through a dehydrating series of alcohols, air-dried overnight and sputtered for 240 seconds with gold with a Denton Vacuum Desk IV. Examinations were done on a Zeiss (Leo) EVO scanning electron microscope based at the Field Museum of Natural History, Chicago.

Results

Live specimens of *O. sinuata* were subjected to several kinds of physical stress twice daily over a four-day period. Animals were removed from the leaf litter in which they were kept and placed in a small, rectangular plastic arena floored with damp paper toweling. Transfer was accomplished using a plastic spoon to avoid disturbing the millipedes. After two minutes of recovery time, the animals began freely exploring the arena. They were tapped lightly with the head of an insect pin, which induced them to roll up as described above. The enrolled animals were then lightly squeezed with padded forceps. It was possible to quickly grasp walking millipedes by one or more legs, which also caused them to enroll, often around the tips of the forceps. Individuals were dropped into small vials and shaken. None of these disturbances caused any visible secretion that could be observed at 20X magnification under a dissecting microscope. In contrast, the secretion of *Glomeris marginata* is easily elicited and clearly visible (Carrell and Eisner 1984, Eisenbeis and Wichard 1987 [their fig. 88b], pers. obs. by TW).

Scanning electron micrographs of the ozopores of glomerids have never been published. As depicted by Verhoeff (1926), the inconspicuous pores are actually located in the intersegmental membranes immediately behind the posterior margins of the diplotergites. There is a slight modification on the anterior margin of the adjacent diplotergite margin near the opening of the gland. These modifications (Figs 3–7), which are more pronounced in *G. marginata* (Fig. 3), were interpreted as helping in the spread of the secretion along the diplotergite (Verhoeff 1926). In our illustrations, the pore itself appears to be double, or at least somewhat constricted in the midline.

GC/MS analysis of the methanol extracts, scanning for a common fragment at *m/z* = 104, revealed the presence of two quinazolinones in a 1:4 ratio (Fig. 1). Their mass spectra (Fig. 2) matched those reported for glomerin and homoglomerin (Meinwald 1966, Schildknecht and Wenneis 1967a). Additionally, a number of fatty acids were detected as their methyl esters (FAMES). Along with the usual C-16 and C-18 FAMES at 20.6 to 21.5 min and 22.7–23.1 min respectively, several C-15 and C-17 FAMES were detected from 19.6 to 20.0 min and 21.6 to 22.1 min respectively (Fig. 1). Based on previous knowledge we assume the quinazolinone alkaloids came from the repugnatorial glands, and the fatty acids likely from fat tissues in the millipedes' bodies.

Discussion

The illustrations in Eisner et al. (1978) are erroneous in showing the pores opening from within the diplotergite. While in their text, Eisner et al. (1978) state that the glands open on segments 4–11, their drawings and photographs clearly show that the glands open just behind segments 3–10. Verhoeff (1928, p. 1030) states, quoting Latzel (1884): "Das erste Saftloch liegt an der Basis des 4. Rückenschildes." If we

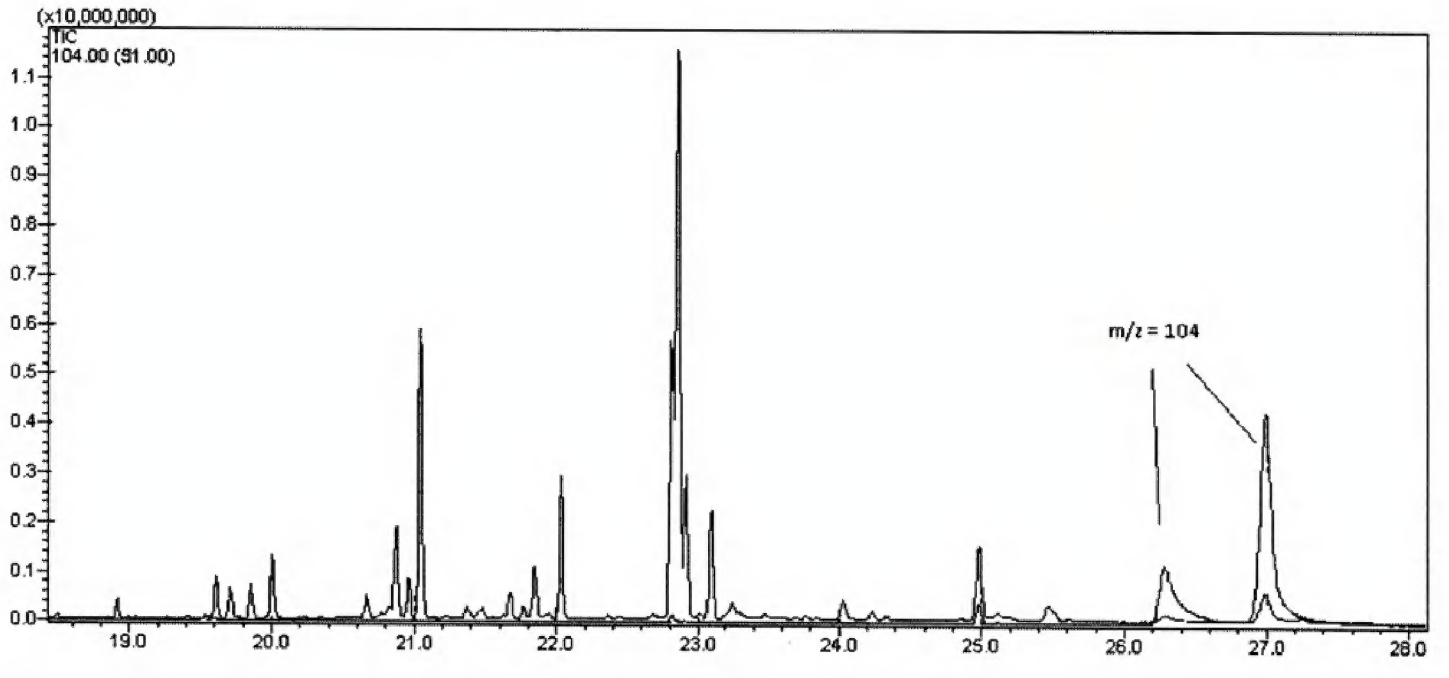


Figure 1. Gas Chromatogram of the extract of *O. sinuata* showing the *m/z* =104 characteristic of Glomerin and Homoglomerin.

take one meaning of "Basis" it is suggested that the pores open *anterior* to the fourth diplotergite, and it is clear from the position of the glands and the modification of the anterior margin of the fourth diplotergite that the glands and the pore actually belong to the third and hence should be considered as opening on segments 3–10, not 4–11. While the illustrated pore of *G. marginata* appears single, the pore of *O. sinuata* seems at least to be constricted in the middle, and that of *Glomeroides primus* (Silvestri, 1929) appears to be bilaterally double, perhaps reflecting the dual nature of the glands. It may be that in the ancestors of the glomerids there were two pores, more lateral in position, serving two separate glands. As the ability to enroll developed the pores and glands may have moved dorsally and partially or entirely fused. Eisenbeis and Wichard (1987) suggest that the middorsal position of the glands is optimal in providing coverage for the enrolled animal. On the other hand, the unique position and structure of the glands, the unusual chemistry of the secretion and its sticky nature, not seen in any helminthomorph millipedes, could be interpreted as supporting a separate evolutionary origin for chemical defense in glomerids. The other two orders of Pentazonia (Glomeridesmida, Sphaeriotheriidae) lack repugnatorial glands and pores.

Given that no secretion could be elicited in the laboratory, we were surprised at the results of the analysis of the extract. Our sample size in the "stress test" is small (4

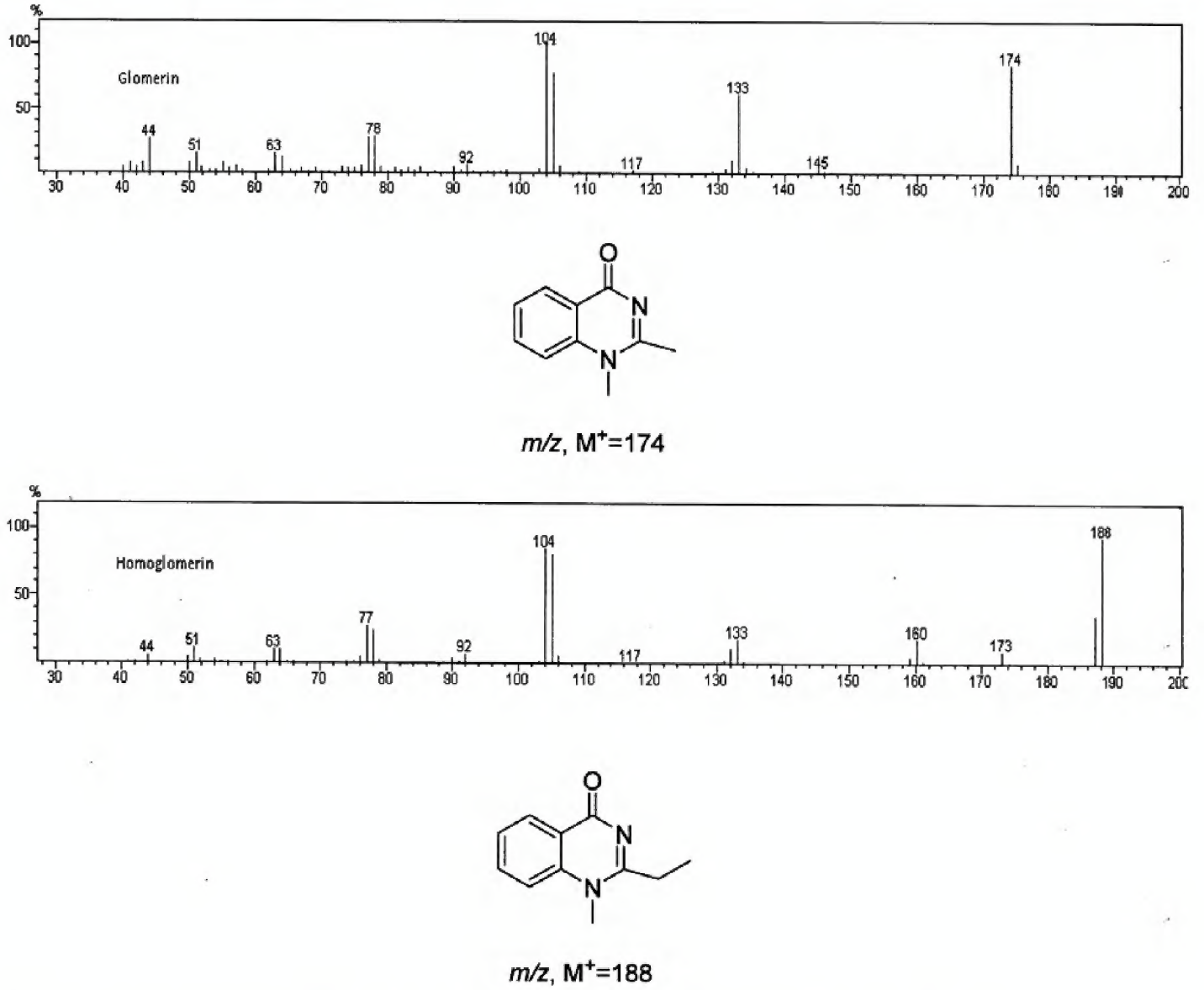
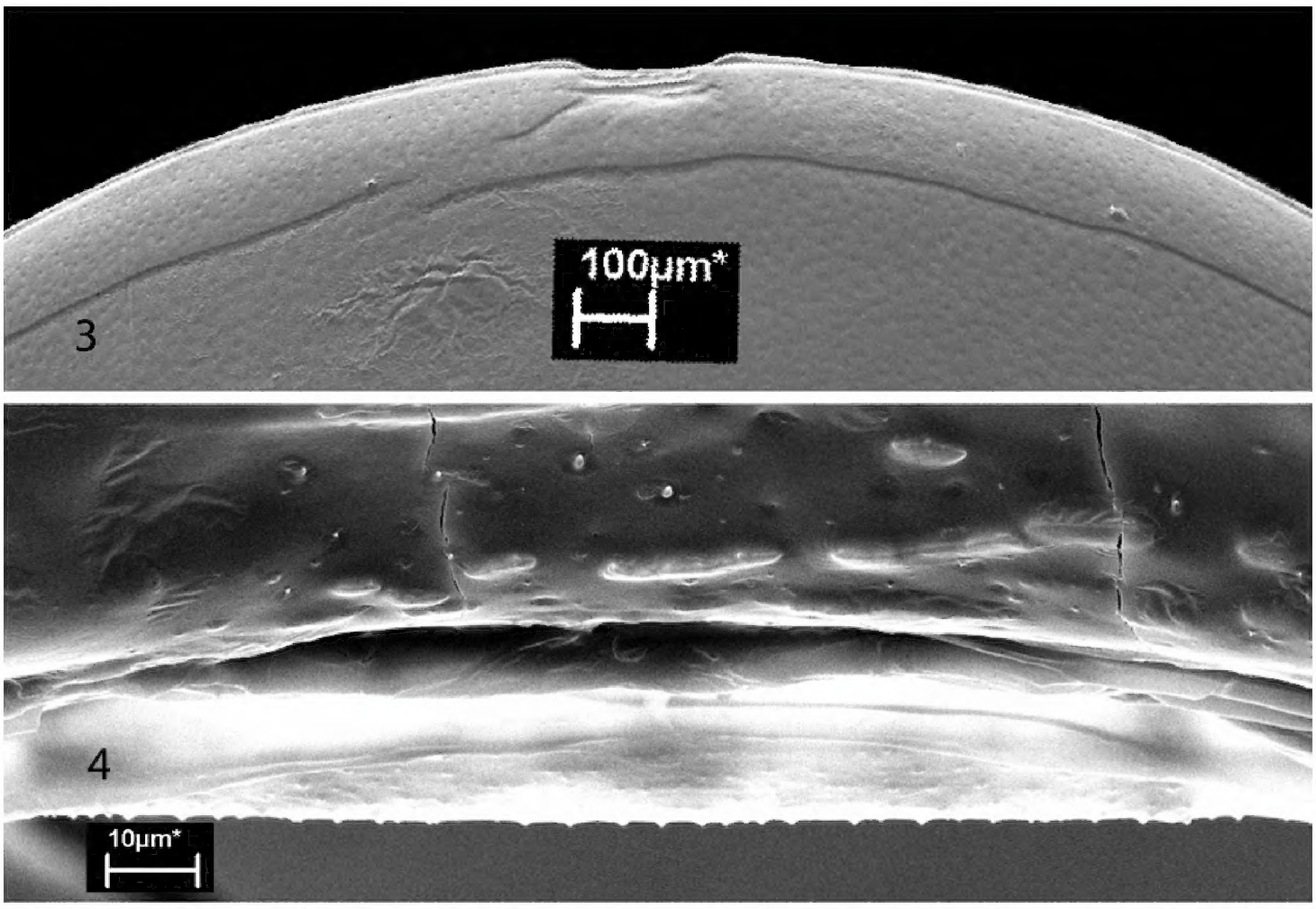


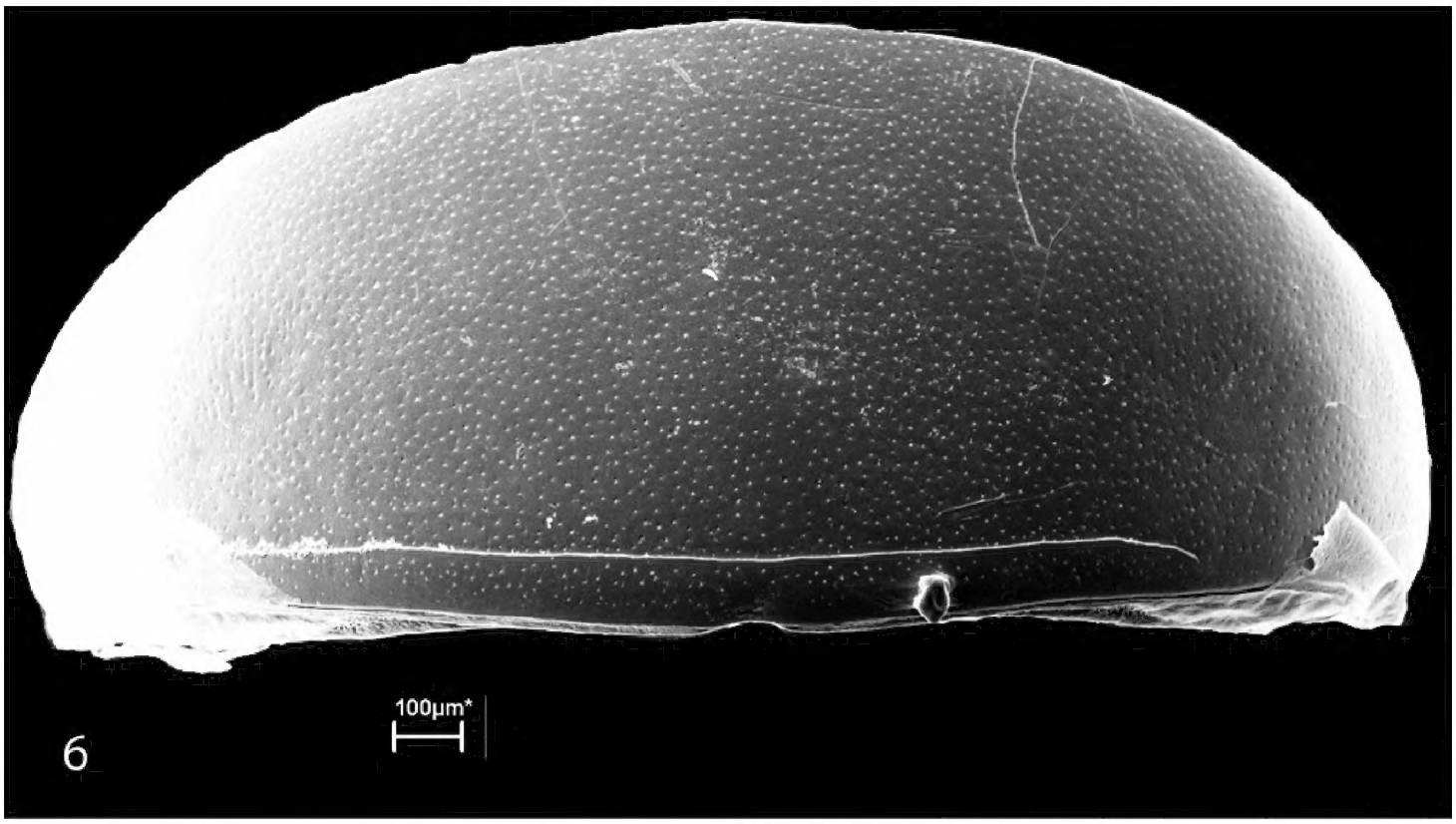
Figure 2. Mass spectra and structures of Glomerin and Homoglomerin respectively.



Figures 3–5. Ozopores of glomerids. **3** Tergite 4 of *Glomeris marginata,* anterior above; note modified tergal margin above ozopore **4** Ozopore of *G. marginata* **5** Ozopore of *Onomeris sinuata;* tergal margin is unmodified.

individuals), but we speculate that some stimulus other than physical disturbance may be needed to trigger the production of secretion. It is likely that ants are major predators of these small, soil and litter-dwelling glomerids, and it may be that some chemical clue associated with ant predation may elicit secretion.

The effectiveness of the quinazolinone alkaloids against ants has never been tested, but the stickiness of the *G. marginata* secretion has been observed to "quickly immobilize" ants (Eisner et al. 2005). However, quinazolinones cause vomiting in toads, partial hypnosis in birds, and partial paralysis in mice (Schildknecht and Wenneis 1967a). Carrell and Eisner (1984) found that glomerin, and especially homoglomerin, were active as antifeedants against wolf spiders, but at a much lower dosage than was needed to completely sedate the spiders of the same size; in fact, spiders which rejected *G. marginata* without killing or feeding on the millipedes often became sedated. That spiders (and perhaps other arthropods) might be the targets of glomerins is suggested



Figures 6–7. Ozopores of *Glomeroides primus.* **6** Tergite 4, anterior below; note modified tergal margin above ozopore **7** Ozopore.

by the fact that closely related compounds such as arborine, a quinazolinone from a plant, and methaqualone, a synthetic sedative, do not sedate spiders (Carrell et al. 1984). Both are sedative to vertebrates (Dey and Chatterjee 1967, Inaba et al. 1973).

However, it is worth noting that *G. marginata* is native to Europe (see Heath et al. 1974 for data on life history), while the spider used in predation tests by Carrell and Eisner (1984) and in sedation studies by Carrell et al. (1984), *Hogna ceratiola* (Gertsch & Wallace, 1935), is endemic to scrub habitats in central Florida, USA. The effects of glomerins have not been tested against potential invertebrate predators of *G. marginata* in its native habitat, though lycosid spiders large enough to attack *G. marginata* occur there.

In keeping with its dark, obscure habitat, *O. sinuata* shows no signs of aposematic coloration and probably is not a target of visually hunting predators, as it rarely appears above the surface of the leaf litter. Our live specimens consistently fled light and concealed themselves in available litter. Aposematism is indicated for many *Glomeris* species, with classic coloration in shiny black offsetting bright yellow, orange or red spots and bands, though *G. marginata* is not obviously aposematic compared to conge-

ners such as *G. humbertiana* Saussure, 1893 or *G. connexa* C.L. Koch, 1847 (Demange 1981, see color plate III). Carrel and Eisner (1984) and Carrell et al. (1984) speculated on the significance of supposed aposematism in *G. marginata.* Typically, aposematic prey survives a predatory attack and the predator becomes "educated" in the avoidance of similar prey. However, *G. marginata* individuals were often killed by *H. ceratiola* spiders before the spider dropped them or became sedated. Further, the sedation would almost always be fatal to the spiders in nature because they would become vulnerable to their own predators, especially to the ever-present ants (Eisner et al. 2005 picture such a spider under attack by ants). Thus Carrell and Eisner questioned how aposematism would work in this system, eventually suggesting that since millipedes have limited mobility, members of the same species in the same area may be related, and therefore the "sacrifice" of some individuals in the population could help relatives that carry some of the same genes. There are two flaws in this scenario. One is that there has been no demonstration that even spiders that survive an encounter with *G. marginata* have been conditioned at all; in fact learning by spiders is very limited. We think it more likely that the rather poorly marked aposematism of *G. marginata* is directed to visually hunting vertebrate predators in its native habitat. The second flaw, of course, is that *H. ceratiola* could not be a natural predator of *G. marginata* because the two species occur an ocean away from each other. Thus tests involving this spider have only a suggestive significance.

More work is required to understand the significance of the presence of glomerin and homoglomerin in *O. sinuata* secretions, especially to determine likely predators of the millipede and the effects, if any, of the secretion against them. We also need to understand why the millipedes seem so reluctant to produce their chemical defense when treated roughly in a way that immediately would cause other species to do so.

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